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THE EVIDENCE FOR THE TRANSFORMATION OF FEMALE FROGLETS INTO MALE ONES FOLLOWING UTERINE OVERMATURATION OF THE EGGS

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THE EVIDENCE FOR THE TRANSFORMATION OF FEMALE FROGLETS INTO MALE ONES FOLLOWING UTERINE OVER-MATURATION OF THE EGGS

A contribution to the critical discussion of the work of K. Wagner*

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ABSTRACT: The author shows that uterine overmaturity of eggs has a sex-transforming effect in frogs.

In his paper on "Experimental investigation of sex transformation in the frog," Wagner comes to results which are in complete contradiction of the results previously obtained. Because of the important role played by overmaturity experiments in the investigation of sex development, it appears exigent to point out that Wagner's conclusions are not based on material facts. Following a critical discussion of his work, I shall avail myself of the occasion to report new evidence pointing to the male-sex-determining effect of egg overmaturity. /168**

I. Previous Findings

a) Statistical evidence. The attempt to prove the occurrence of sex shifts by statistical means represents an obvious approach. This avenue of approach, however, also is the most difficult one since the following requirements must be met in order to permit factual demonstration:

1) One normal control and one culture containing overmature eggs must be grown, both cultures being derived from material of the same genotype.

2) Culture conditions for both cultures must be identical and must come as close as possible to optimal conditions in order to exclude secondary effects.

3) Mortality must be low so that the differences in sex ratios obtained in the two cultures cannot be explained by this factor.

4) The numbers must be large enough to be statistically meaningful.

These requirements have to date been met only in a number of cultures of R. Hertwig and in one additional one of Kuschakewitsch. In the latter one, these

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*Arch. f. Entw. -Mech. 52:386-394, 1923.

**Numbers in the margin indicate pagination in the foreign text.

requirements were met almost perfectly, and I present his findings once more in Table 1.

TABLE 1

	♂	♀	⚥	Total Number	Mortality
Normal series.	58	53	—	111	6%
Overmature series. . .	299	—	1	300	4%

b) Biogenetic evidence. A second form of demonstration may be arrived /169
at by biogenetic investigations, i.e., by the demonstration of the occurrence of a transformation of ovaries into testes. This form of demonstration is complicated by the fact that the males of the common frog strains undergo an indirect testicular development. Sex transformation in animals derived from overmature eggs must, therefore, be demonstrated specifically. This is done either by the comparison with controls or by the statistical demonstration that a sex transformation in females has taken place. My culture of overmature eggs No. D20 (Witschi 1914a and 1914b[1]) may serve as an example.

TABLE 2

Age	♂ + ⚥	♀	Intersex
13—17 days	—	—	10
24 "	2	—	2
28—42 "	22	20	8
Metamorphosis			
51 days	4	1	—
52—97 "	46	—	—

Total number of larvae and frogs: 115
Mortality in larvae and frogs: 35%

Overmaturity of the eggs amounted in this instance to 80-100 hours. The mating couples belonged to the undifferentiated Irschenhausen strain. As may be seen in Table 2 sex differentiation was first noted on the 17th day of development. Despite the fact that these animals belonged to an undifferentiated strain, a number of males appeared first. This finding is indicative for the effect of overmaturity as is the arrest of a number of gonads in the indifferent stage up to the moment of metamorphosis. These, however, represent minor findings, which will not be further discussed in this connection. The important fact is that the equilibrium between the sexes was established at the moment of metamorphosis (which occurred between the 30th and 41st day). Transformation of females into males commences immediately following this event. On the 51st day already, female animals disappeared from entry in the table. Our material, however, must be regarded as /170

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[1] A list of papers published on this subject between 1912 and 1922 may be found in my review in Zeitschr. f. indukt. Abstammungs- u. Verebungslehre 31, 1923.

being too small in numbers—and this in particular because of the relatively high mortality—to permit application of the results obtained as definite proof for sex transformation. But the animals no longer shown in the columns showing the number of females can be demonstrated to exist as hermaphrodites. In the table these intersex forms are shown together with the males because the gonads of these latter forms exhibit a continuous series of transformational stages, the last one of which exhibits the picture of an entirely testicular tissue. Thus, for instance, the gonads of the 13 froglets fixed on day 97 exhibited in two cases a chiefly ovarial, in three cases a chiefly testicular, in seven cases a purely masculine, and in the final case—one is tempted to say—and ultramasculine character. In this latter case, the testes exhibited a peculiar hypotrophic picture, which is characteristic for severe damages due to overmaturity. Details on the transformation process observed in the gonads of one of these 97-day-old froglets have appeared elsewhere (Witschi, E.: Arch. f. Entw.-Mech. 49, 1921; Figs. 6-8, In Table 7). The figures exhibited there show ovarial tissue of this intersex gland undergoing degeneration with simultaneous proliferation of spermatogenetic tissue and formation of testicular ampullae. The microscopic examination, thus, confirmed that a sex transformation in the direction from female to male sex was taking place, and this finding confers conclusive force on the results obtained in the statistical investigation of such changes occurring during development.

II. K. Wagner's Investigations

a) With respect to the statistical aspects, none of the four requirements listed above appear to have been met.

1. Not only was no control series instituted, but of the four culture series with overmature eggs, only two each had been fertilized by the same male. Thus, strictly speaking, only two of these series can be compared with one another. In connection with the question under discussion, this particular detail may be disregarded since the genotypically identical series exhibit the same degree of overmaturity (72 and 96 hours, respectively).

2. The culture conditions were not identical, since the experiment on overmaturity was combined with a feeding experiment. On the basis of the protracted and irregular development of the larvae (the average in the four cultures being 72, 79, 78 and 109 days, respectively), one may conclude that breeding conditions were unfavorable.

3. Average mortality was 77%, and this fact makes a presentation of conclusive proof impossible.

4. Finally, the material was numerically too small since the four cultures yielded for final examination only 24, 8, 27 and 23 animals, respectively.

Surprisingly, Wagner did not conclude his statistical evaluation. If he had done so, the results obtained would have shown that retardation of fertilization by 24 hours caused a doubling of the number of males (from 9% to 18%), and this at the expense of the number of intersex individuals (cf. Table 3). Once one realizes that his investigation was terminated at the moment of beginning metamorphosis, then /171

TABLE 3

Length of overmaturity	72 hours		96 hours	
♀	19	60%	30	60%
⚥	10	31%	11	22%
♂	3	9%	9	18%

the result just pointed out confirms the one obtained by me and discussed further above. His result also agrees well with that obtained by R. Hertwig (1921) in his latest investigation, which demonstrated that differentiation in the males of undifferentiated strains is accelerated by a slight degree of overmaturity. If Wagner had continued his experiment for a number of months beyond metamorphosis, he would probably also have noted the occurrence of transformation of females.

b) As I have mentioned above, Wagner failed to draw these statistical conclusions. He, instead, attempted a biogenetic examination of the question. This form of examination appears to be impossible a priori since his material was not fixed in series. His cultures, however, were of different ages at the moment of metamorphosis: The larvae of three series underwent metamorphosis at the age of 72 - 79 days, and those of the fourth one after 109 days of breeding. It is, however, not permissible to regard the first three cultures as representing the earlier, and the latter one as representing the later stage, as has been done by Wagner. A 72-day-old larva undergoing metamorphosis can in no way be regarded as representing an intermediate form of a 109-day-old larva also just undergoing metamorphosis. The thesis that the development of the ovaries and their products in the frog is independent from that of the body is based on error. On the contrary, both factors are closely correlated. There exist, however, factors which simultaneously cause a retardation of metamorphosis and enlargement of gonads, as, for instance, is the case with exposure to cold (Witschi 1922b). A similar factor must have been the cause for Wagner's observation of the marked increase in size of the ovaries exhibited by animals belonging to his 109-day-series.

Table 4 exhibits Wagner's material in the manner he arranged it. The increase in the number of females in the last series cannot be regarded as being significant because of the small total number and in particular not because the number of males also increased. Striking is only the almost complete absence of intersex individuals. Wagner interprets this finding by assuming that the intersex forms have been transformed into females, and he believes to have demonstrated that "sex transformation in the frog in the course of development may also proceed in the direction from male to female." This conclusion is a very bold one indeed since evidence that sex transformation in fact has occurred in his series is completely missing. Not only did he not demonstrate any transformed animals but there also is no deviation at all from the normal ratios found by Wagner in animals captured in the field (Females: males 2:1 to 5:1). /172

TABLE 4

Development	72, 79, 78 days		109 days	
♀	31	53%	18	78%
⚥	20	34%	1	4%
♂	8	14%	4	17%

The situation demonstrated in Table 4 might be more easily understood by applying the following considerations. As mentioned above, the 109-day-series, with its protracted development and the marked increase in size of the ovaries exhibited by the animals of this series, bears a certain resemblance to a cold culture. This particular culture is furthermore characterized by a marked retardation of differentiation of the male sex. The absence of intersex individuals in this aberrant series should therefore be regarded as indicating that the transformation of ovarial into testicular tissue had as yet not commenced. The effect of overmaturity is inhibited by the same factor which causes retardation of development. If this is correct, then this culture must, of course, be eliminated from the evaluation of the effects of overmaturity. Only the three first cultures, which developed at approximately the same rate, may be used for comparison. Table 3 should therefore be replaced by Table 5. This table shows that the series containing eggs fertilized late exhibits a very considerable increase of the male tendency at the expense of the females.

As explained further above, it is impossible to demonstrate any fact at all with Wagner's data. I believe, however, that I have been able to show that a proper evaluation of his material indicates that the results obtained by him are rather in agreement than in disagreement with the results obtained in previous experiments.

TABLE 5

Length of overmaturity	72 hours		96 hours	
♀	19	60%	12	44%
⚥	10	31%	10	37%
♂	3	9%	5	19%

The histologic examination of the gonads of the intersex forms revealed nothing new. The figures show that the material examined represents an early stage of transformation from a female gonad into a male one, with insufficient development of female germinal epithelium, as one frequently finds in cultures of overmature eggs. /173

c) A further point concerning Wagner's paper deserves discussion viz. the determination of the degree of overmaturity. Wagner obtained overmature material by separating freshly captured mating partners and fertilizing the eggs yielded after an interval of 72 and 96 hours, respectively. He then regards as certain the result "that 100% of males do not necessarily develop in all instances from highly overmature uterine Rana fusca eggs." Why does the author state that these eggs were highly overmature? Hertwig and Kuschakewitsch never estimated overmaturity from the time of capture of the copulating partners but from the moment of the first, normal deposition of eggs. At which time this event occurred in Wagner's mating frogs cannot be ascertained. Common frogs normally remain for days in copula. In instances of premature copulation, eggs situated in the uterus are occasionally even days later not yet fully matured. On the other hand, it is possible in certain instances that eggs attain overmaturity while still in the ovary. This uncertainty in the determination of the degree of maturity makes experimentation, of course, very difficult, and this in particular in the experimentation with common frogs. On the basis of the experimental results obtained by Wagner, his 72-hour- and 96-hour-series give the impression of containing slightly and moderately strongly overmature eggs, respectively.

In a preliminary communication, I (1922c) stated that it is possible to determine the degree of overmaturity by examining the cleavage pattern of the eggs. This pattern is characterized by the presence of small micromeres at the 8-celled stage and by a number of other special features. It is thus possible to obtain information on the degree of maturity of the eggs employed six hours after fertilization. Last year I have checked all my hybridization series by this method.

Finally I may remark that a yield of "100% of males" in a large culture will always represent a gift of chance. In almost all cases one will find that the eggs of one overmature spawn are affected to different degrees. Frequently one part of the eggs is no longer viable, while another part produces normally metamorphizing females. This erratic distribution of the effects of overmaturity may even be observed in individual eggs, and frequently one finds that the development of certain regions of one egg is retarded at the cleavage stage already. The embryo shown in Fig. 2, thus, exhibits a particularly severe lesion of the right posterior quarter of the body. Experience has taught us that germinal cells are highly sensitive toward overmaturity. One may, however, accept as certain that somatic defects occasionally occur without changes taking place in the gonads at the same time.

In the next part of this paper I shall describe the examination of a number /174
of animals partially damaged due to overmaturity of the eggs.

III Recent Investigations

In two previous papers, I have reported on the teratogenetic significance of overmaturity (1920; 1922c). Double and multiple formations of legs and the anterior part of the body represented the most frequently occurring and most striking malformations caused by this factor.

The possibility of recognizing the effects of overmaturity in individuals by examining both changes in the cleavage pattern and external malformations has opened a new avenue for the investigation of these effects on sex distribution.

In the spring of 1922, I prepared the following fertilization series. I divided the eggs of one Davos female into four groups (designed "Z") and fertilized them individually with the sperm of three males—Davos ("z"), Alsace ("e"), and Rostock ("g")—and with the sperm of an intersex individual from Freiburg i. Brsg. ("f"), which animal genetically was a female. I thus obtained the parallel cultures Zz, Ze, Zg and Zf. As previously reported in a paper on this series[1], I recognized at the 8-celled stage that the eggs employed were overmature. The cultures, however, were not discarded. Because of the inclusion of the intersex animal, these cultures simply were too valuable to be abandoned, and, furthermore, the degree of overmaturity was a very small one. Figure 1 represents a sketch from my

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[1]"Über die genetische Konstitution der Froschzwitter" (On the genetic constitution of intersex frogs), Biol. Zbl. 43, 1923.

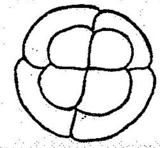

Figure 1. Egg at the 8-celled stage, as shown in the protocol on the progeny of the female "Z". The micromeres are smaller than under normal conditions, which fact indicates overmaturity of the egg.

Figure 2. Photograph of a 1-1/2-day-old frog embryo obtained from a culture of overmature eggs (V"). The right posterior side is dying off.

protocols, and it shows the marked decrease in the size of the micromeres. A few other eggs were even more atypical, while a great number of eggs, on the other hand, were entirely normal. I thus expected only a minor deviation from the normal sex distribution, i.e., from results controlled by the genetic make-up provided. This expectation was borne out by the results obtained. The four cultures will be discussed briefly and the possible effects of overmaturity will be examined.

A. The Numerical Sex Ratio in Cultures Zz, Ze, Zg and Zf /175

a) Zz (♀ Davos × ♂ Davos).

Since the Davos as well as other Alpine frog strains in all normal combinations have been shown to be differentiated strains, a normal sex ratio was expected in accordance with the genetic make-up of the animals. This experiment yielded 149 females and 141 males, i.e., a close approximation to the ratio of 1:1. No marked sex ratio shift was found.

Overmaturity, however, produced certain effects in one single froglet, which animal is not included in the numbers given above. This animal exhibited partial external lesions viz. a bud consisting of two rudimentary limbs had developed from the thoracic girdle (Fig. 7).

b) Ze (♀ Davos × ♂ Alsace).

The genetic investigation of the animals of the Alsace strain has shown them to be a mixture of forms differentiated to varying degrees. This hybridization experiment yielded a small excess of females, i.e., 150 females and 123 males.

The male employed for fertilization obviously belonged to a strain exhibiting moderate to strong differentiation. Effects of overmaturity were not noted in this series.

c) Zg (♀ Davos × ♂ Rostock).

The Rostock strain is one exhibiting moderately strong differentiation. The number of females is therefore somewhat greater in this series, i.e., 170 females and 122 males were obtained. This result, too, is due entirely to genetic factors, and it is not influenced by overmaturity. This culture, like the Zz-culture, yielded however one animal exhibiting malformations due to overmaturity of the egg viz. a simple duplication of the left forelimb. The hand of this extra limb interestingly exhibited five fingers (Fig. 3).

d) Zf (♀ Davos × ⚥ Freiburg).

This last combination is of particular interest. Since the hermaphrodite "f" most probably genetically was a female (cf. my study cited above), the entire progeny in this culture exhibited a female constitution. In fact, this culture yielded 237 females (99%). Two animals (1%), however, exhibited the effects of overmaturity of the eggs. The first of these two animals exhibited characteristic testes, and thus was a normal male animal. The appearance of this male in this particular culture unequivocally demonstrates that sex transformation—and this in the direction from female to male sex—is, in fact, possible.

The second froglet showing the effects of overmaturity was characterized by an external lesion viz. the right hindlimb had developed into a stunted rudiment (Fig. 10). Since the abdomen on the same side also was underdeveloped, this animal must have developed from an egg affected in a similar manner as the /176
egg which produced the embryo shown in Fig. 2.

B. The Sex of Froglets Exhibiting Malformation due to Overmaturity of the Egg

The internal picture exhibited by the three froglets affected by overmaturity obtained in cultures Zz, Zg and Zf, respectively, corresponded in a marked manner to the external picture of malformation.

a) Kidneys. Figures 3, 7 and 10 show that the external malformations are accompanied by defects of corresponding parts of the kidneys. In the first case (Fig. 3) only the anterior pole of the left kidney was affected by underdevelopment. In the second case (Fig. 7), the anterior poles of both kidneys were underdeveloped, the left one more so than the right one. This difference corresponded to the more ventral position of the supernumerary limb exhibited by that animal. Figures 8 and 9 show cross sections through the underdeveloped kidney parts. Uriniferous tubuli are completely absent; these are replaced by a sparse renal blastema.

The third case (Fig. 10) exhibited a total reduction of the right kidney. The cross section exhibits only few blastema rudiments (Nbl.) with interspersed chromaffin cells (Ch; Fig. 11). /177

Figure 3. Photograph of a froglet undergoing metamorphosis obtained from culture "Zg". One supernumerary forelimb, a kidney diminished in size, and a rudimentary gonad may be seen on the left side. Threefold magnification.

b) Gonads. In parallel to the findings obtained in the kidneys, the gonads also remained underdeveloped. This fact is very clearly shown in the animal of the Zf-series shown in Fig. 10, where the right gonad is represented by a short, transparent, spindle-shaped body. The left gonad of the second animal of the Zz-series is almost as stunted as that of the former (Fig. 7). The right gonad in the posterior part, however, is of almost normal size. This gonad exhibited a normal length, but it tapered off toward the anterior part, and the tissue covering /178
the rudimentary kidney region had lost its ivory color and had become bluish transparent (which fact indicates a deficient development of the germinal epithelium). The gonads of the froglet obtained in the Zg-culture (Fig. 3) exhibited the best development. The left gland, however, was again underdeveloped, while the right one was well formed. In the posterior part, this gonad exhibited a definitely ovarial character; in the anterior third, the gland again tapered off to a marked degree, and it again had lost its ivory color.

c) Sexual funiculi. The poor development shown by the gonads in any case is the direct consequence of the precarious condition of the kidneys or the renal blastema, respectively. The condition of these tissues, in turn, is responsible for the poor development exhibited by the sexual funiculi, which are of great importance for the development of the gonads, and in particular for that of the male gonads. The rudimentary condition of the sexual funicuculus (S) is exhibited in the gonadal section shown in Fig. 11 (Zf-series). The development of this funiculus corresponds to that of a normal, 10-day-old, 18 mm long larva. The funiculi in the left gonad of the Zz-animal are markedly stronger. As may be seen in Fig. 9, the funiculus (S) is exposed to much better growth condition in the renal blastema present, in this instance, in ample amounts. The funiculi gain normal size in all those cases where there are normal kidneys lying under the gonads. This, for instance, is the case in the right side of the Zg-animal (Figs 4 - 6).

d) Sex. All three animals genetically represent females, a fact made evident by the demonstration of the ovarial character of the gonadal regions lying outside of the regions affected by overmaturity just described. In all three instances, however, sex transformation had commenced in the parts modified by overmaturity. Normal testicular development, however, is found only under circumstances where the sexual funiculi have the chance to develop correctly. This, for instance, is the case in the right gonad of the Zg-animal. In successive sections one may—proceeding from the posterior to the anterior part—find all transitional stages from characteristic

ovarial to characteristic testicular tissue. Figure 4 shows a microphotograph of a section through the posterior half of the gonad, exhibiting the characteristic structure of an ovary of a freshly metamorphosed common frog. The sexual funiculus (S) exhibits a short compact end in the mesovarium, and it represents the endothelium of the large secondary genital cavity, or ovarial pocket (O), in the /179
center of the gonad. The germinal epithelium contains relatively numerous oogonia (Ov) in the outer border region. In the basal zone, i.e., in the region where the germinal epithelium changes into the mesovarium, the oogonia retain their undifferentiated character for a longer period of time, while maturation of the ovum begins early in the distal parts of this tissue. In these regions of the sec- /180
tion one may see clusters of eggs in the synaptene (syn) and pachytene (pach) stages of development, respectively, as well as a number of free auxocytes (A).

In the region of transition into the anterior third of the gonad, one finds a fully developed process of transformation taking place. Figure 5 shows one of the earliest stages. The rough outlines of ovarial germinal epithelium can still be discerned. Maturation of the eggs is discontinued, and the youngest oocyte stages are absent (up to the synaptene stage). Many structural elements are undergoing degeneration, and in the distal parts in particular one may see germinal epithelium undergoing lysis. The most characteristic changes may, however, be found in the sexual funiculus. The endothelium of the ovarial pocket—the lumen of which has been almost completely compressed—by a process of extensive proliferation and redifferentiation has changed into a compact funiculus representing the anlage for the rete testis. In earlier papers I have repeatedly reported on the important role played in sex transformation by the undifferentiated oocytes lying at the gonadal base (Fig. 4). In most cases, these particular oocytes are transformed into spermatogonia in the course of the transformation process. Germinal cells in other regions of the ovary much more rarely undergo this form of transformation. Figure 5 shows that a connection between the basal ends of the germinal epithelium and the sexual funiculus is established by extensive fusion. On the left (medial side) the oocyte is still firmly implanted in the germinal epithelium; on the right, however, two germinal cells (Sp) have joined the sexual funiculs, and these cells must now be regarded as representing spermatogonia.

Figure 6 shows a section through the middle of the anterior third of the gonad. The development in the direction toward male tissue must have commenced much earlier in this part since this tissue exhibits a purely testicular character; ovarial rudiments are not present. In the middle, one may see the sexual funiculus exhibiting the characteristic structure of a rete testis of this stage of development, and around the rete one may find seminiferous ampullae (Amp) in the process of formation. The plain peritoneum is all that is left of the germinal epithelium.

In the left gonad of this animal as well as in the rudimentary gonads of the two other ones, this clear picture of sex transformation is obliterated greatly by the insufficient development of both the kidneys and the sexual funiculi. Figure 8 shows a section through the anterior part of the right gonad of the Zg-animal (Fig. 7). The germinal epithelium is poorly developed and it contains undifferentiated germinal cells only. One germinal cell has already migrated into the proliferating sexual funiculus, but it is developing in the form of a "giant

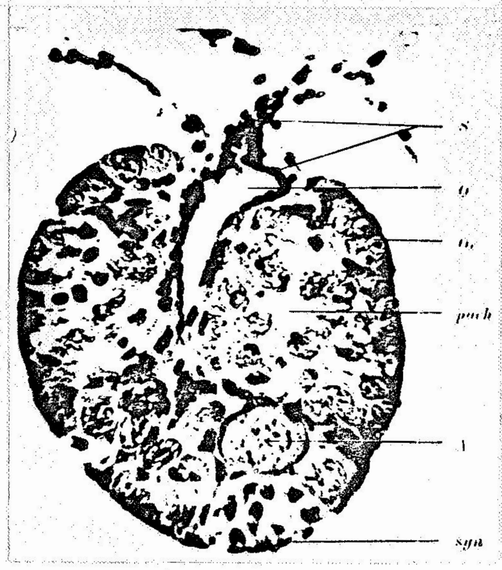

Figure 4.

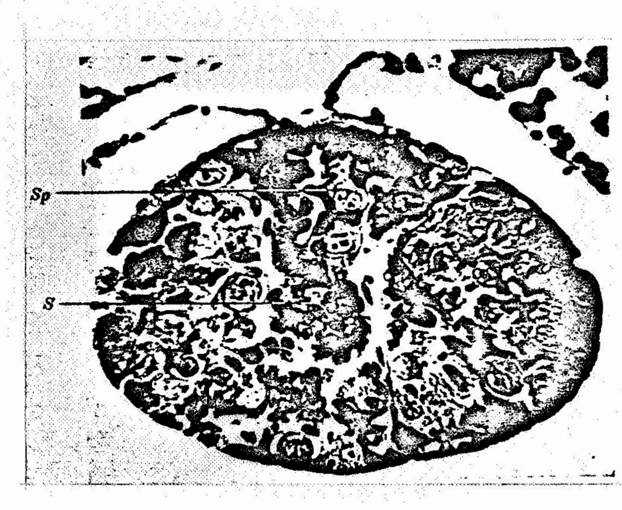

Figure 5.

Figures 4 - 6. Microphotographs of three cross sections through the right gonad of the froglet shown in Fig. 3. The posterior part of the gonad exhibits a purely female character (Fig. 4). Sex transformation sets in just before the middle line of the gland (Fig. 5). The anterior end exhibits a pure masculine character (Fig. 6). S: sexual funiculus; O: ovarial pocket; Ov: oogonium; syn: egg cluster with synaptene oocytes; pach: egg cluster with pachytene oocytes; A: oocytes in the early stages of development (auxocytes); Amp; seminiferous ampullae; P: peritoneum. 300-fold magnification.

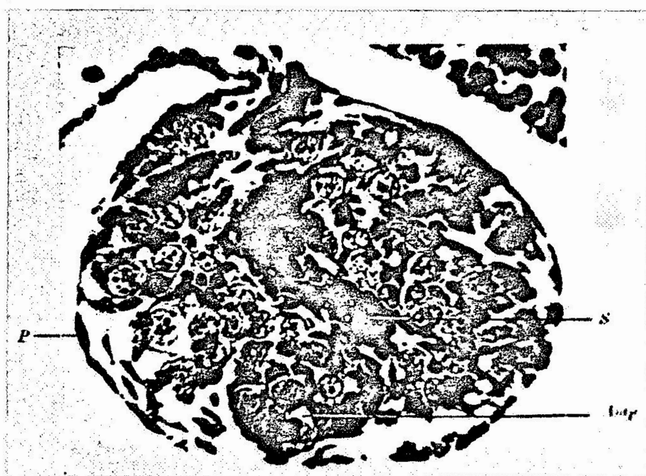

Figure 6.

spermatogonium" in an atypical manner (Rsp). This finding probably represents a form of cellular hermaphorodism.

Figure 9 shows that the left gonad of the same animal exhibits a picture corresponding to the early stages of testicular development, one that is normally found in 12-day-old, 22 mm long larvae. From the renal blastema, the sexual funiculus grows into the primary genital space (p. G.), and the germinal cells as yet undifferentiated, forming long chains, stretch out toward the proliferating ends of the funiculus. /182

Figure 7. Photograph of a froglet undergoing metamorphosis obtained from culture "Zz". The left side exhibits a "parasitic" malformation with two rudimentary limbs. The left gonad is severely, the right one less severely underdeveloped. The anterior kidney ends are rudimentary. Threefold magnification.

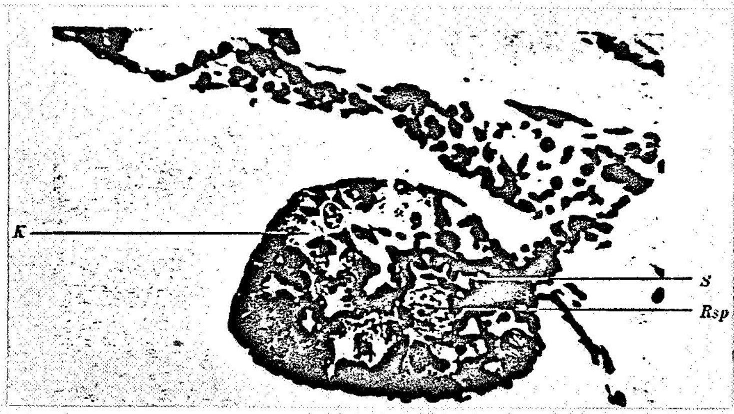

Figure 8. Microphotograph of a section through the anterior part of the right gonad of the animal shown in Fig. 7. The germinal epithelium (K) is poorly developed. The sexual funiculus (S) is proliferating. One germinal cell (Rsp) has migrated into the tissue of the funiculus. 300-fold magnification.

The still very small gonad of the Zf-animal (Figs. 10 and 11) represent the lowest stage of testicular formation. A decisive male tendency is indicated by the penetration of the undifferentiated germinal cells into the gonadal interior as well as by the arrest of the germinal cells in the undifferentiated stage, which is characteristic for spermatogonia. In view of the almost complete absence of sexual funiculi, actual testicular formation is as yet impossible in this instance.

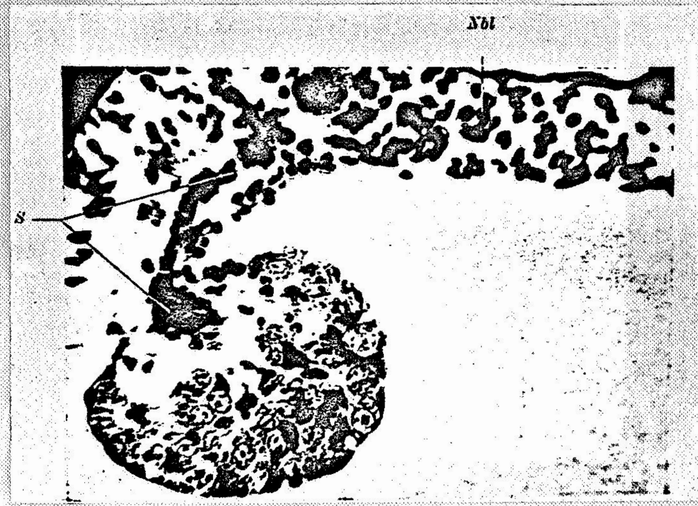

Figure 9. Microphotograph of a section through the left rudimentary gonad of the same animal. The germinal epithelium contains a thich layer of undifferentiated germinal cells, which are growing toward the proliferating end of the sexual funiculus (S). Instead of a kidney, only blastema cells (Nbl) may be found 300-fold magnification.

Figure 10. Photograph of a froglet undergoing metamorphosis obtained from culture "Zf". The right hindlimb is severely stunted as is the right gonad. The right kidney is very small. Threefold magnification.

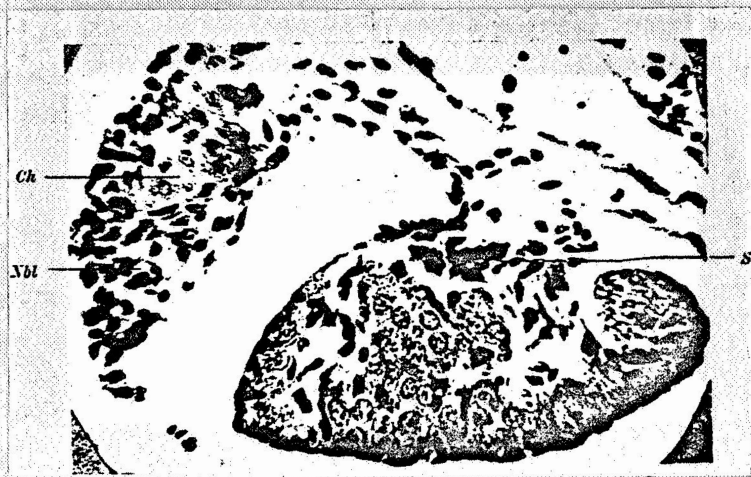

Figure 11. Microphotograph of a section through the right gonad of animal in Fig. 10. The renal blastema (Nbl) insignificant, containing, however, chromaffine cells (Ch) of the suprarenal gland medullae. 300-fold magnification.

C. Summary and Conclusions

1. A part of the eggs of the Davos female "Z" exhibited at the 8-celled stage characteristic deviations from the normal cleavage pattern, from which fact a low-grade overmaturity may be concluded.

2. Effects of overmaturity during metamorphosis were observed in only one animal each of cultures Zz and Zg, and in two animals of culture Zf. The general capacity for development and the sex ratio was otherwise not noteworthily affected in these four cultures.

3. In the three animals exhibiting external defects due to overmaturity, examination of the internal organs demonstrated the existence of corresponding changes in these organs viz.

   a) A rudimentary state of the kidneys;

   b) inferior development of the gonads;

   c) a rudimentary state of the sexual funiculi; and

   d) sex transformation in the direction from female to male individual.

4. Since the regional parallelism of sex transformation in the gonads and the somatic lesions mentioned indicate the existence of a common cause, and since overmaturity has been shown to represent the cause of the characteristic external malformations, the findings obtained represent a new demonstration of the sex-transforming effect of overmaturity.

5. The findings demonstrated furthermore show that the effects of uterine overmaturity on sex distribution are not due to changes in the mechanism of the second reduction division or to selective fertilization. In these three instances, we are without doubt dealing with a metagenetic effect exerted on the germinal cells, which effect does not set in before the stage of gonadal anlage, because we know that the germinal cells are originally situated in the entoderm of the embryo in the form of an azygous medial vitelline streak (Witschi 1914a). In the /183 course of development these cells traverse the closing mesenterium and form an azygous germinal cell funiculus at the dorsal root of the mesenterium. This funiculus is split to the same extent that the principal veins unite to form the lower vena cava; the germinal cells move apart in lateral direction until they reach the proximity of the mesonephric ducts, where the gonadal anlagen are then formed in the peritoneal folds. The fact that sex transformation in the partially damaged animals commenced in the region of the affected mesodermal organs demonstrates that this transformation does not proceed from the germinal cells but is induced by the somatic environment.

The findings described represent an instructive example of an influence exerted on sex distribution by internal factors with a regionally limited space of action.

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